

HAUER et al.  
S.N. 10/031,695  
OA November 30, 2005

**AMENDMENTS TO THE CLAIMS:**

1. (withdrawn) A modified cytochrome P450 monooxygenase which, in comparison with the wild-type enzyme, shows an altered substrate profile in the terminal and/or subterminal enzymatic hydroxylation of aliphatic carboxylic acids, owing to site-specific mutagenesis of its substrate binding region.
2. (withdrawn) A monooxygenase as claimed in claim 1, which is derived from cytochrome P450 monooxygenases of bacterial origin.
3. (withdrawn) A monooxygenase as claimed in claim 2, which is derived from *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 with an amino acid sequence in accordance with SEQ ID NO:2, which has at least one functional mutation in one of the following amino acid sequence regions: 24-28, 45-51, 70-72, 73-82, 86-88, 172-224 and 352-356, with the proviso that, if the enzyme carries the mutation FS7A, more than one of these regions is mutated.
4. (withdrawn) A monooxygenase as claimed in claim 3, which comprises at least one functional mutation in the amino acid sequence regions 86-88 and 172-224.
5. (withdrawn) A monooxygenase as claimed in claim 4, which comprises at least one of the following amino acid substitution patterns:
  - a) F87V;
  - b) F87A L188K;
  - c) F87V L188K;
  - d) F87A L188 KA74G;
  - e) F87V L188K A74G;
  - f) F87A L188K A74G R47F;
  - g) F87V L188K A74G R47F;
  - h) F87A L188K A74G R47F V26T; or
  - i) F87V L188K A74G R47F V26T;and functional equivalents thereof.

HAUER et al.  
S.N. 10/031,695  
OA November 30, 2005

6. (withdrawn) A monooxygenase as claimed in claim 3, which comprises a single amino acid substitution from amongst the following:
  - a) V26T,
  - b) R47F,
  - c) S72G,
  - d) A74G,
  - e) F87V,
  - f) L188z, where Z is an amino acid selected from amongst K, R, W, Q, N, G, A and S, and
  - g) M354T;and functional equivalents thereof.
7. (withdrawn) A nucleic acid sequence encoding a monooxygenase as claimed in claim 1 and the complementary nucleic acid sequence thereof.
8. (withdrawn) An expression construct comprising, under the genetic control of regulatory acid sequence, an encoding sequence which encompasses a nucleic acid sequence as claimed in claim 7.
9. (withdrawn) A vector which encompasses at least one expression construct as claimed in claim 8.
10. (withdrawn) A recombinant microorganism which has been transformed with at least one vector as claimed in claim 9.
11. (withdrawn) A microorganism as claimed in claim 10, selected from amongst bacteria of the genus *Escherichia*.
12. (currently amended) A process for the enzymatic production of subterminally hydroxylated aliphatic carboxylic acids, which comprises

HAUER et al.  
S.N. 10/031,695  
OA November 30, 2005

- a1) culturing a recombinant microorganism which has been transformed with a vector which encompasses an expression construct comprising, under the genetic control of regulatory nucleic acid sequences, a sequence which encompasses a nucleic acid sequence encoding a monooxygenase which is derived from *Bacillus megaterium* cytochrome P450 monooxygenase BM-3 with an amino acid sequence ~~in accordance with~~ of SEQ ID NO:2 which has a functional mutation in the amino acid sequence region 86-88 and optionally at least one further functional mutation in one of the following amino acid sequence regions: 24-28, 45-51, 73-82 and 172-224 with the proviso that, if the enzyme carries mutation F87A, more than one of these regions is mutated, which functional mutation in comparison with the wild-type enzyme, results in an altered activity or regioselectivity in the subterminal enzymatic hydroxylation of, an aliphatic C<sub>8</sub>-C<sub>12</sub>-carboxylic acid, whereby culturing is performed in the presence of a culture medium which contains at least one hydroxylatable C<sub>8</sub>-C<sub>12</sub>-carboxylic acid or a derivative thereof, said derivative being selected from ~~at~~ an alkyl ester, an amide or an anhydride thereof; or
- a2) incubating a reaction medium containing at least one hydroxylatable C<sub>8</sub>-C<sub>12</sub>-carboxylic acid or a derivative thereof, said derivative being selected from an alkyl ester, an amide or an anhydride thereof with a modified monooxygenase as defined above, and
- b) isolating the resulting hydroxylated product from the medium.

13. (canceled)

14. (currently amended) A method as claimed in claim 12, wherein the hydroxylatable carboxylic acid is a C<sub>8</sub>-C<sub>12</sub>-monocarboxylic acid or a derivative thereof and the monooxygenase comprises at least one of the following amino acid substitution patterns in an amino acid sequence ~~according to~~ of SEQ ID NO:2:

- a) F87V;

HAUER et al.  
S.N. 10/031,695  
OA November 30, 2005

- b) F87A and L188K;
  - c) FS7V and L188K
  - d) F87A L188K and A74G;
  - e) F87V L188K and A74G;
  - f) F87A L188K ~~and~~ A74G and R47F;
  - g) F87V L188K ~~and~~ A74G and R47F;
  - h) F87A L188K A74G R47F and V26T; or
  - i) F87V L188K A74G R47F and V26T.
15. (canceled)
16. (previously presented) A method as claimed in claim 12, wherein the enzymatic production is carried out in the presence of an electron donor or a reduction equivalent.
17. (previously presented) A method as claimed in claim 16, wherein the electron donor or the reduction equivalent is selected from amongst NADH, NADPH and Zn/CO(III) sepulchrate.
18. (previously presented) The process of claim 12, wherein the monooxygenase comprises at least one functional mutation in the amino acid sequence regions 86-88 and 172-224.
19. (new) The process of claim 12, wherein the monooxygenase comprises at least one functional mutation in the amino acid sequence regions 86-88 and 172-224.